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ROLE OF THE CORPORA CARDIACA IN THE BEHAVIOR OF SATURNIID MOTHS. II. OVIPOSITION

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Mating in the case of many insects serves as a pivotal event in the life of the adult female. This is especially true for the non-feeding Lepidoptera such as silkmoths of the family Saturniidae. In these insects one can define two conditions, the virgin state and the mated state, each with its own behavioral and physiological characteristics. A case-in-point is the silkmoth, *Hyalophora cecropia*. Here the virgin female assumes a "calling" posture for sex pheromone release at a specific time of the night (Riddiford and Williams, 1971). This behavior is terminated once mating takes place (Falls, 1933). Moreover, once mating has taken place oviposition is stimulated and longevity is reduced (Rau and Rau, 1914). Due to its quantitative nature we have chosen to study the rate of oviposition as an index of the change from the virgin to the mated state.

Materials and Methods

1. *Experimental animals*

The experiments were performed on the silkmoth *Hyalophora cecropia*. Diapausing pupae were obtained from Nebraska and were chilled for at least 12 weeks at 5° C prior to use.

2. *Excision of the corpora allata and corpora cardiaca*

The removal of the corpora allata or the corpora allata-corpora cardiaca complexes was performed as described by Williams (1959).

3. *Castrations*

Pupae were anesthetized with CO_2 and placed dorsal-side up in a plasticine cradle. A square of cuticle was excised from the dorsal midline of the third abdominal segment, and the underlying heart was pushed to the side of the operating field. The pupal testes, which are situated in the dorso-lateral region of the fourth abdominal segment, were removed with Dumont #5 forceps. A few crystals of phenylthiourea and streptomycin (Williams, 1959) were placed in the wound, and the operated area was covered by a piece of plastic cover-slip which was sealed in place with melted wax. To prevent the pupa from dislodging the seal, the fourth abdominal segment was immobilized by placing melted wax between segments 3 and 4. After 4 to 5 weeks at 25° C the castrated pupae gave rise to apparently normal moths which mated and produced well-formed, albeit sterile spermatophores.

4. *Matings of the moths*

The females were mated on the first or second day after their emergence. Unoperated females were placed with males in a 1 × 1 × 1 foot cage in the evening. The next morning non-mating animals were removed from the cage and stored elsewhere. The mating pairs were separated in the late afternoon.

In order to assure that all operated females mated, these females were hand-mated by a technique developed by C. M. Williams (personal communication). The female was deeply anesthetized with CO_2. An unanesthetized male moth was grasped by the wings and the genitalia were stimulated with a camel's hair brush to initiate the opening of the claspers. The tip of the female's abdomen was exposed by gentle pressure on the abdomen and brought into contact with the male genitalia. In most cases the males readily clasped the female and copulation ensued. The pair were separated 12 to 20 hours later.

The success of a mating to an unoperated male was judged by the production of fertile eggs. A successful mating to a castrated male was indicated by the remains of a spermatophore in the female's bursa copulatrix.

5. *Collection of eggs*

Each female was placed in a large paper bag. The number of eggs deposited each day was counted during each of the next six to seven days. At the end of this period, the females were sacrificed and dissected under Ringer's solution. The number of chorionated eggs remaining in the ovarioles was determined. This number was added to the total laid by each female and the latter expressed as a percentage of the total number of eggs that had matured.

In the graphs of the oviposition patterns of mated moths, the data are referenced to the number of days after the termination of mating. In the case of unmated females, the end of day 1 was scored when the first 5 or more eggs had been oviposited. In most cases this occurred the third or fourth day after emergence.

Results

1. *The effect of mating on the pattern of oviposition*

Figure 1A summarizes the cumulative per cent of eggs oviposited by 12 virgin females over a period of 6 days. Oviposition occurred at essentially a constant rate of 7% per day. After 6 days an average of 40% had been deposited and 60% retained.

To assess the effect of mating, 26 females were mated to unoperated males. Twenty-four of these crosses were fertile. The pattern of oviposition of these twenty-four is shown in Figure 1B. On the first day after mating the percentage of eggs laid by mated females was nearly as great as the total laid by virgin females over the entire six-day period. After six days, most females retained fewer than a dozen eggs in their ovarioles. This pattern of oviposition is essentially identical to that which Taschenberg and Roelofs (1970) reported for mated Ceropia.

The two unsuccessful matings were also of interest. Although the remains of a spermatophore were present in the bursa of both females, none of the eggs

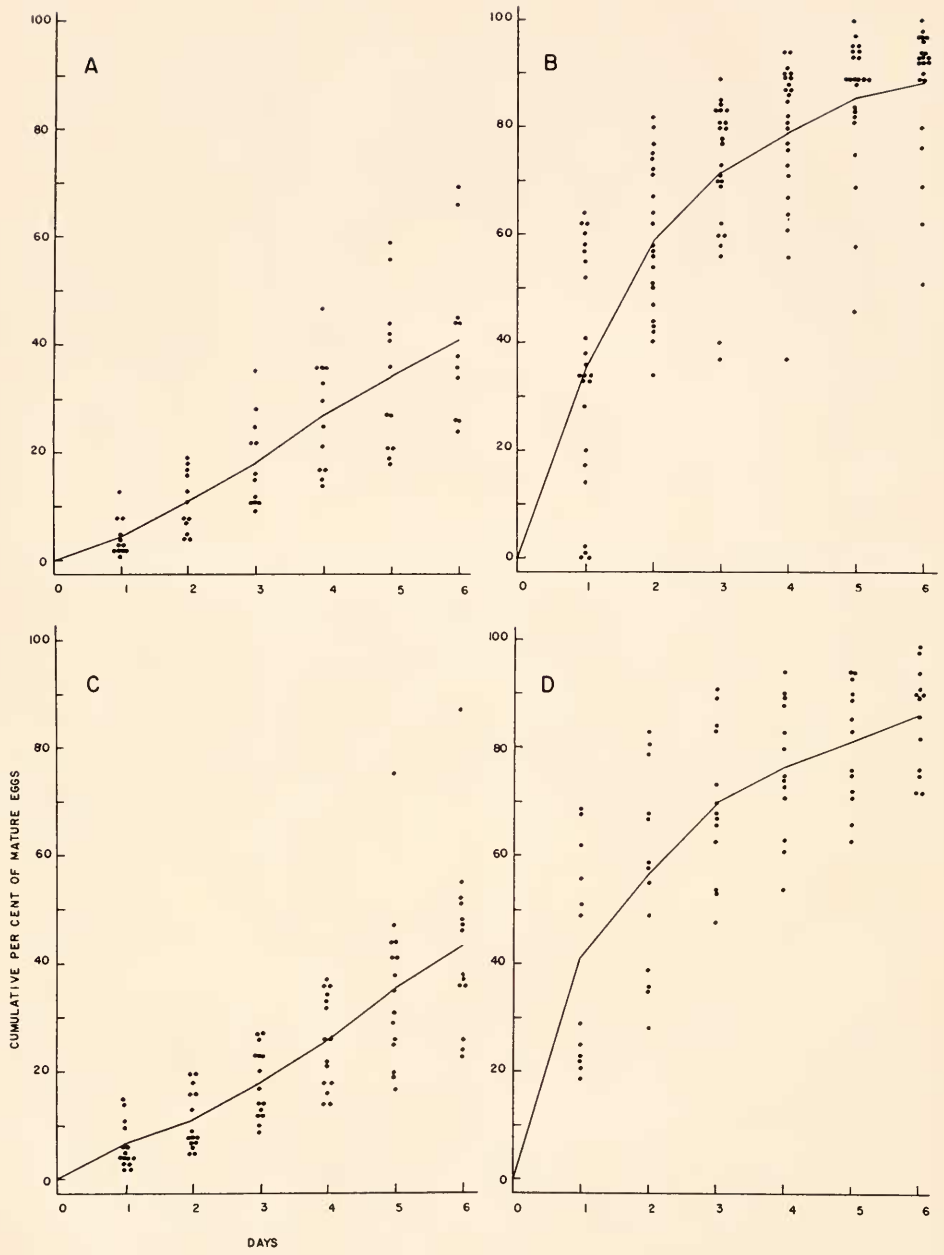

FIGURE 1. The oviposition patterns of Cecropia females: A., virgin females; B., females successfully mated to normal males; C., females successfully mated to castrated males; D., allatectomized females successfully mated to normal males.

was fertile. Both individuals showed a pattern of oviposition similar to that of virgin females.

2. *Matings to castrated males*

Fifteen females were mated to castrate males. As shown in Figure 1C, the pattern of oviposition was indistinguishable from that of virgin females. Upon autopsy, each female showed the remains of a spermatophore in the bursa copulatrix. Autopsy of the castrated males revealed normally developed accessory glands which were full of secretory products. Consequently, it was assumed that the spermatophores were normal except for the absence of sperm.

3. *Removal of the corpora allata*

The excision of the corpora allata does not interfere with oogenesis (Williams, 1959), or with the production and release of sex pheromone (Riddiford and Williams, 1971) by female Cecropia moths. However, there remained the possibility that the corpora allata might be involved in the switchover from virgin to mated behavior in response to insemination. To test this possibility the corpora allata were removed from thirteen female pupae. The pupae were then placed at 25° C and allowed to develop into female moths. The females were hand-mated to normal moths. As seen in Figure 1D, the removal of the corpora allata did not significantly interfere with the response of these females to insemination.

4. *Removal of the complex of corpora allata and corpora cardiaca*

The corpora allata-corpora cardiaca complexes were removed from 21 female pupae. Into the thorax of four of these pupae were reimplanted 4 or 5 pairs of "loose" corpora allata-corpora cardiaca complexes. A total of 14 of the resulting female moths were successfully mated to normal males (three of these had received implants of the complexes).

The removal of the complex of corpora allata and corpora cardiaca essentially abolished the response to insemination (Fig. 2A). As in the case of virgin females, the rate of oviposition was constant over a six-day period; the average rate was about 10% per day as compared to 7% for virgin females. The implantation of up to five pairs of complexes had no effect in restoring the mated response (Fig. 2A, open circles).

Seven females lacking corpora allata and corpora cardiaca were mated either to castrated males or to normal males without successful insemination. All seven showed the same type of behavior as unoperated females under the same conditions—*i.e.*, the virgin pattern of oviposition (Fig. 2B).

Discussion

1. *The stimulus for the change in female behavior*

In most insects mating stimulates oogenesis and provokes an abrupt change in the behavior of the female (Norris, 1933). The specific stimuli which effect these changes are variable. In cockroaches the mechanical stimulus of the spermatophore in the bursa copulatrix brings about the mated response (Roth and Stay,

1961). In *Drosophila* (Kummer, 1960) and mosquitoes (Leahy and Craig, 1965) the transfer to the female of a secretion from the male accessory gland is responsible for the change. In the Lepidoptera (Klatt, 1920; Norris, 1933) and Hemiptera (Davey, 1965) the presence of sperm in the female spermatheca is apparently the trigger.

The present findings on Cecropia are in substantial agreement with those reported for other Lepidoptera. Matings with castrated males involved the passage of a sterile spermatophore but did not cause the switch in behavior. Consequently, the presence of sperm itself appears to be the stimulus which triggers the transformation of behavior to the mated pattern.

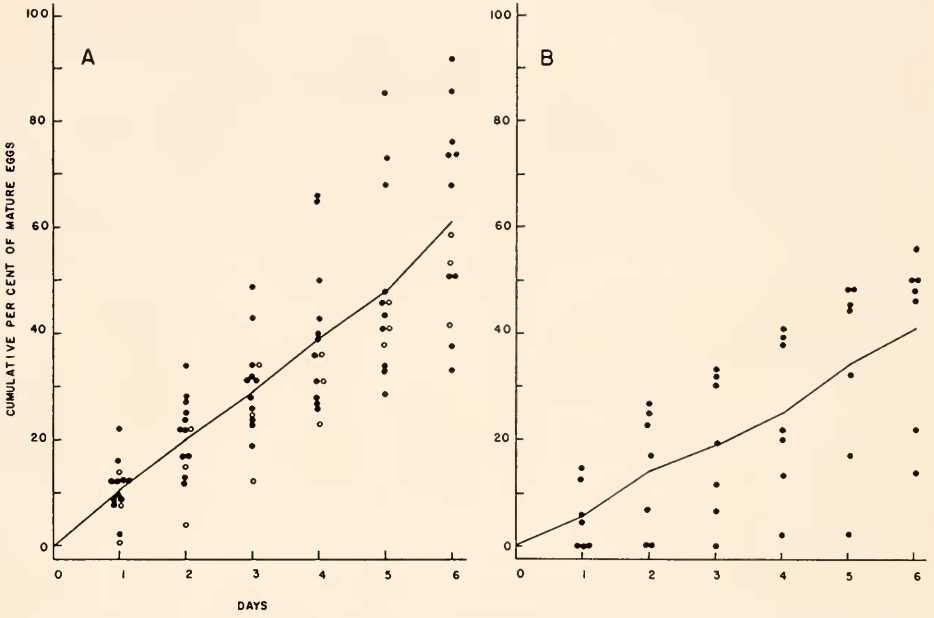

FIGURE 2. The oviposition patterns of allatectomized-cardiectomized Cecropia females: A., females successfully mated to normal males—the open dots refer to those which had 5 pairs of allata-cardiaca complexes reimplanted; B, females mated to castrated males or unsuccessfully mated to normal males.

2. *The endocrine relay: the corpora cardiaca*

The effect of mating on oogenesis is presumably mediated by the corpora allata (Engelmann, 1968). But in the case of the behavioral change, the involvement of the endocrine system has remained unexplored. The saturniids have proven to be convenient animals on which to study the change in oviposition behavior because in these insects egg maturation is independent of the corpora allata and the female moths emerge with essentially a full complement of eggs. Thus one circumvents any complications arising from a dependence of oviposition on oogenesis.

As seen in the Results, the corpora cardiaca, but not the corpora allata are necessary for the behavioral change. After removal of the corpora allata-corpora

cardiaca complexes, mating resulted in only a slight elevation in the oviposition rate over that of virgin females. This was in marked contrast to the results obtained by mating unoperated or allatectomized females to normal males. The fact that the implantation of up to five pairs of complexes into cardiacectomized females did not restore the oviposition response shows the necessity of intact connections between the brain and corpora cardiaca. Therefore, the relationship is identical to that controlling pheromone release by these moths (Riddiford and Williams, 1971). The intrinsic cells of the corpora cardiaca apparently produce an oviposition stimulating factor, and the release of this factor is controlled by the brain.

It is important to note that the unsuccessful matings of allatectomized-cardiacectomized females gave the same result as seen in virgin females or in females which were mated to castrate males. From this we learn that the removal of the corpora cardiaca does not interfere with the process of egg-laying itself, but only with the increase in oviposition rate which is provoked by insemination.

3. *The role of the corpora cardiaca*

The corpora cardiaca appear to have a central role in controlling the behavior of the female moth. In the virgin condition the brain directs the release of a "calling" hormone from this organ (Riddiford and Williams, 1971). The results reported here indicate that after the reception of sperm by the female, the brain ceases to promote "calling" and now stimulates the release of an oviposition hormone from the corpora cardiaca. Consequently, the corpora cardiaca serve as the switch by which the brain changes the behavior of a female from the virgin to the mated condition.

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Summary

1. In females of the Cecropia silkmoth, mating stimulates the rate of egg deposition.

2. When virgin Cecropia are mated to castrated males, they receive an apparently normal spermatophore except that it lacks sperm. Such females show an oviposition pattern corresponding to that of unmated females. Thus, the normal increase in oviposition rate is triggered by the reception of sperm by the female.

3. The corpora cardiaca are shown to be involved in this change in oviposition rate. After the corpora allata are removed from female pupae, the resulting moths respond normally to insemination. After removal of the corpora allata-corpora cardiaca complexes, the ovipositional response to mating is effectively abolished. Reimplantation of up to 5 pairs of corpora allata-corpora cardiaca complexes into pupae lacking their own complexes does not restore the normal oviposition pattern.

4. It is concluded that the increase in oviposition rate is due to a hormone which is produced by the intrinsic cells of the corpora cardiaca. By an apparent reflex mechanism, the presence of sperm in the female's spermatheca causes the brain to trigger the release of the hormone in question from the corpora cardiaca.

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